

## MODULE 3                      RIVERS AND STREAMS

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**3.1.**

**AMBIENT BIOLOGICAL MONITORING**

## AMBIENT BIOLOGICAL MONITORING

As part of the SWAT program, the Biological Monitoring Unit evaluates benthic macroinvertebrate communities of Maine streams and rivers to determine if they are impaired by toxic contamination. For reasons of comparability, a small number of unimpaired reference sites is also evaluated. Benthic macroinvertebrates are animals without backbones that can be seen with the naked eye and live on the stream bottom, such as mayflies, stoneflies, caddisflies, crayfish, snails, and leeches. In 2006, we evaluated the condition of 39 sample locations, primarily in the Penobscot River and North Coastal Rivers basins.

The Biological Monitoring Unit uses a multivariate statistical model to analyze a benthic macroinvertebrate sample and predict if a waterbody is attaining the biological criteria associated with its statutory class. If a waterbody does not meet minimum state aquatic life criteria, Class C, then the model class is predicted as Non-Attainment (NA). Classes AA and A are treated the same in the model. Final decisions on aquatic life attainment of a waterbody are made accounting for factors that may allow adjustments to the model outcome. This is called the final determination.

Table 3.1.1 summarizes the results of biological monitoring activities for the 2006 SWAT Program, sorted by waterbody name. Column headings of Table 3.1.1 are described below:

- *Station* – Since waterbodies are sometimes sampled in more than one location, each sampling location is assigned a unique “Station” number.
- *Log* – Each sample event is assigned a unique “Log” number.
- *Map* – The “Map” number refers to Maps 1 through 29, which are located after the tables.
- *Location* – Some Stations are located upstream or downstream of potential sources of pollution, which are called “Issues”.
- *Issue* – Issues are potential sources of pollution.
- *Statutory Class* – The state legislature has assigned a statutory class, either AA, A, B, or C, to every Maine stream and river. Class AA and A waterbodies shall support a “natural” biological community. Class B waterbodies shall not display “detrimental changes in the resident biological community”. Class C waterbodies shall “maintain the structure and function of the resident biological community”.
- *Final determination* – The final decision on aquatic life attainment of a waterbody. Accounts for factors that may allow adjustments to the model outcome.
- *Attains Class* – “Y” is given if the final determination is equal to or exceeds the Statutory Class. A Class B stream, for example, would receive a “Y” if its Final determination was either A or B. “N” is given if a stream does not attain its Statutory Class. A Class B stream, for example, would receive an “N” if its final determination was either C or NA. A dash (“-”) is given if the sample was disturbed or provided insufficient information.
- *Probable Cause* – The probable cause column lists potential stressors to benthic macroinvertebrate communities, based on best professional judgement. In some cases, a probable cause may not be related to toxic pollution but instead to poor habitat conditions.

Data reports for each sampling event (Aquatic Life Classification Attainment Reports) are available in electronic format with the web version of this report. Supporting water chemistry data are given in Table 3.1.2. Water temperature data are given in Figure 3.1.1. For more

information about the Biological Monitoring Unit, please e-mail us at [biome@maine.gov](mailto:biome@maine.gov) or visit our web site: <http://www.state.me.us/dep/blwq/docmonitoring/biomonitoring/index.htm>.

## Results Summary

- Thirty-nine stations were assessed for the condition of the benthic macroinvertebrate community.
- Results have been received to date (June 12, 2007) for twenty-three stations.
- Eleven of the twenty-three stations (48 %) reported failed to attain the aquatic life standards of their assigned class.

## Historical Notes

(not all of the samples listed below were collected under the SWAT Program)

- Birch Stream (Station 312) failed to attain class in 1997, 1999, 2001, 2003, 2004, and 2005.
- Dennys River (Station 297) failed to attain class in 1997, 1999, and 2003.
- East Machias River (Station 494) failed to attain class in 2001.
- Great Falls Branch (Station 504) failed to attain class in 2001.
- Millinocket Stream (Station 287) attained class in 1996.
- Mopang Stream (Station 501) attained class in 2001.
- Narraguagus River (Station 81) failed to attain class in 1984 and 1993. It attained class in 2001.
- Narraguagus River (Station 111) attained class in 1987 and 2001. It failed to attain class in 1989 and 1996.
- Narraguagus River (Station 112) attained class in 1987.
- Penjajawoc Stream (Station 314) failed to attain class in 1997, 2001, 2002, and 2003.
- Penjajawoc Stream (Station 315) attained class in 1997 and 2001. It failed to attain class in 2002 and 2003.
- Penjajawoc Stream (Station 511) failed to attain class in 2001, 2002, and 2003 .
- Penobscot River (Station 62) attained class in 1984, 1993, 1994.
- Piscataquis River (Station 83) attained class in 1984, 1985, 1989, 1996. It failed to attain class in 1990.
- Piscataquis River (Station 135) attained class in 1989, 1990, and 1996.
- Piscataquis River (Station 152) attained class in 1991, 1993, and 1995.
- St. Croix River (Station 199) attained class in 1991 and 1997.
- Shaw Brook (Station 480) failed to attain class in 2001.
- Sheepscot River (Station 74) attained class in 1987, 1989, 1990, 1992, 1995, 1996, 1998, 1999, 2000, 2001, 2002, 2003, 2004, and 2005. It failed to attain class in 1984, 1985, 1986, 1988, 1991, 1993, 1994, and 1997.
- Souadabscook Stream (Station 290) attained class in 1996.
- Souadabscook Stream (Station 291) attained class in 1996.
- Unnamed Stream 2 (Station 633) failed to attain class in 2002.
- Unnamed Stream 4 (Station 634) attained class in 2002.
- West Branch Sheepscot River (Station 268) attained class in 1995, 1996, 1997, 1998, 1999, 2001, 2002, and 2005. It failed to attain class in 2000, 2003, and 2004.

**TABLE 3.1.1 - 2006 SWAT Benthic Macroinvertebrate Biomonitoring Results**

Name	Town	Map	Station	Log	Location	Issue <sup>1</sup>	Statutory Class/ Final Determination	Attains Class?	Probable Cause <sup>1</sup>
Birch Stream	Bangor	1	312	1549	Down-stream	Urban NPS; Airport	B / NA	N	NPS toxics; habitat
Card Brook	Ellsworth	2	814	1547		Urban NPS	B / NA	N	Habitat
Card Brook	Ellsworth	2	815	1548		Urban NPS	B / NA	N	NPS toxics; habitat
Dennys River	Meddybemps	3	297	1585		Haz Waste/ Liming	AA / A	Y	BPJ
Dennys River	Dennysville	4	741	1582			AA /		
E. Br. Penobscot River	T3R7 WELS	5	823	1589		Reference	AA /		
East Machias River	Crawford	6	494	1586			AA /		
Garland Pond Outlet	Sebec	7	817	1568			B /		
Great Falls Branch	Deblois	8	504	1579		Agric NPS	A /		
Jepson Brook	Lewiston	9	824	1592		Urban	B / NA	N	NPS toxics; habitat
Kenduskeag Stream	Bangor	1	829	1550		Urban NPS	C / B	Y	
Little River	Columbia Falls	10	821	1581		Reference	A / A	Y	
Little Smith Brook	Millinocket	11	819	1572			A / C	N	Habitat
Millinocket Stream	Millinocket	11	287	1571		Urban NPS	B /		
Moose Brook	Auburn	12	816	1562			B /		
Mopang Stream	T30 MD BPP	13	501	1587			AA / A	Y	
Narraguagus River	Cherryfield	14	81	1576		Agric NPS	B /		
Narraguagus River	Deblois	8	111	1577		Agric NPS	AA /		
Narraguagus River	Beddington	15	112	1578		Reference	AA /		

<sup>1</sup> NPS = non-point source pollution; Haz waste = hazardous waste; Agric NPS = agricultural NPS; BPJ = Best Professional Judgment

**TABLE 3.2.1 - 2006 SWAT Benthic Macroinvertebrate Biomonitoring Results (cont.)**

Name	Town	Map	Station	Log	Location	Issue <sup>1</sup>	Statutory Class/ Final Determination	Attains Class?	Probable Cause <sup>1</sup>
Penjawoc Stream	Bangor	16	314	1556		Urban NPS	B / C	N	NPS toxics; habitat
Penjawoc Stream	Bangor	16	315	1557		Urban NPS	B / C	N	NPS toxics; habitat
Penjawoc Stream	Bangor	16	511	1555		Urban NPS	B / C	N	NPS toxics; habitat
Penobscot River	Orono	17	62	1552		Munic/Ind	B / A	Y	
Piscataquis River	Abbot	18	83	1565		Reference	A / A	Y	
Piscataquis River	Sangerville	19	135	1566		Munic/Ind	B /		
Piscataquis River	Dover-Foxcroft	20	152	1567		Munic/Ind	B /		
St. Croix River	Baring	21	199	1584		Munic/Ind	C / A	Y	
Sebec River	Milo	22	827	1569		Municipal	B /		
Seboeis Stream	Howland	23	665	1575			A / A	Y	
Shaw Brook	Hermon	24	480	1551		Urban NPS	B / NA	N	NPS toxics; habitat
Sheepscot River	North Whitefield	25	74	1539		Reference	AA / A	Y	BPJ
Souadabscook Stream	Hampden	24	290	1553		Landfill	A / A	Y	BPJ
Souadabscook Stream	Hampden	24	291	1554		Landfill	A / A	Y	BPJ
Unnamed St. 2	Topsham	26	633	1564		Urban NPS	B / A	Y	
Unnamed St. 4	Topsham	26	634	1563		Urban NPS	B /		
W. Br. Sheepscot River	China	27	268	1540		Reference	AA / B	N	
Wassataquoik Stream	T3R7 WELS	28	812	1590		Reference	AA /		
West Seboeis Stream	T4 R9	29	818	1570			A /		
Western Little River	Columbia	10	820	1580			AA / C	N	

<sup>1</sup> NPS = non-point source pollution; Munic/Ind = municipal/industrial; BPJ = Best Professional Judgment

**TABLE 3.1.2 - 2006 SWAT Nutrients and Solids Data**

Log	Waterbody	Sampling Date	DOC	NH <sub>3</sub> -N	TKN	NO <sub>2</sub> -NO <sub>3</sub> -N	O-PO <sub>4</sub>	Total P	TSS	TDS
			mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1549	Birch Stream	8/9/2006	4.2	0.03	0.3	0.32	0.006	0.022	~1.3	360
1548	Card Brook	8/8/2006	19	0.03	0.8	0.02	0.003	0.031	5	170
1550	Kenduskeag Stream	8/9/2006	11	0.01	0.5	0.07	0.008	0.032	2	100
1571	Millinocket Stream	8/16/2006	4.4	<0.01	0.2	0.01	0.002	0.008	~1.7	21
1576	Narraguagus River	8/21/2006	7.6	0.01	0.3	0.02	0.001	0.015	~1	32
1578	Narraguagus River	8/21/2006	7.7	<0.01	0.3	<0.01	0.001	0.012	0.8	32
1555	Penjajawoc Stream	8/10/2006	12	0.01	0.7	<0.01	0.003	0.029	4	90
1556	Penjajawoc Stream	8/10/2006	11	0.01	0.7	0.02	0.004	0.025	3	150
1557	Penjajawoc Stream	8/10/2006	12	0.01	0.6	0.02	0.003	0.021	~1.6	180
1552	Penobscot River	8/9/2006	11	<0.01	0.4	0.03	0.002	0.013	~0.7	45
1565	Piscataquis River	8/15/2006	5.5	<0.01	0.2	0.02	0.001	0.019	10	24
1566	Piscataquis River	8/15/2006	5.1	0.01	0.2	0.05	0.004	0.016	~1.4	27
1567	Piscataquis River	8/15/2006	5.7	0.01	0.3	0.03	0.002	0.013	~1.4	50
1575	Seboeis Stream	8/17/2006	7	<0.01	0.3	<0.01	0.001	0.009	~0.8	<20
1551	Shaw Brook	8/9/2006	6.7	0.02	0.4	0.18	0.002	0.032	10	230
1539	Sheepscot River	8/7/2006	11	0.01	0.5	0.02	0.003	0.014	~1	50
1553	Souadabscook Stream	8/10/2006	7.8	0.03	0.6	0.01	0.007	0.032	~1	70
1554	Souadabscook Stream	8/10/2006	18	0.01	0.5	0.02	0.008	0.030	2	70
1564	Unnamed Stream 2	8/14/2006	1.9	0.03	0.2	0.89	0.003	0.015	3	150
1563	Unnamed Stream 4	8/14/2006	2.2	0.08	0.2	0.45	0.002	0.015	8	370
1563	Unnamed Stream 4 DUPLICATE	8/14/2006	2.2	0.08	0.3	0.45	0.002	0.018	8	380
1540	W. Br. Sheepscot River	8/7/2006	9.6	0.01	0.5	0.03	0.002	0.013	~1	60
1540	W. Br. Sheepscot R. DUPLICATE	8/7/2006	9.3	0.01	0.5	0.03	0.002	0.015	~1.3	60

DOC = dissolved organic carbon, NH<sub>3</sub>-N = ammonia-nitrogen, TKN = total Kjeldahl-nitrogen, NO<sub>2</sub>-NO<sub>3</sub>-N = nitrite-nitrate-nitrogen, O-PO<sub>4</sub> = Ortho-phosphate, Total P = total phosphorus, TSS = total suspended solids, and TDS = total dissolved solids.

More detail including maps of sampling stations, temperature data, and raw macroinvertebrate data are available in the Ambient Biological Monitoring section on our website at <http://www.state.me.us/dep/blwq/docmonitoring/swat/index.htm>



**3.2**

**FISH CONSUMPTION ADVISORIES**

## COPLANAR PCB

In 2006 the SWAT program was again integrated with the Dioxin Monitoring Program (DMP) that has been in effect since 1988. Fish samples collected at 15 DMP stations for dioxin analyses were also analyzed for coplanar PCBs in the SWAT program. All non-detects were calculated at half the detection limit. Dioxin toxic equivalents (DTEh) and coplanar PCB toxic equivalents (CTEh) were calculated using World Health Organization (1998) toxicity equivalency factors (TEFs). For comparison with the Maine Center for Disease Control and Prevention's (MCDC) (formerly Maine Bureau of Health) Fish Tissue Action Levels (FTAL) for protection of human consumers, the 95<sup>th</sup> upper confidence limits (95% UCL) were used. The 95%UCL DTEh are compared to the cancer action level, FTALc=1.5 ppt, and the 95%UCL TTEh (sum of both CTEh and DTEh) are compared to the reproductive and developmental action level, FTALr=1.8 ppt and both are compared against the potentially lower fish tissue action level (pFTAL=0.4 ppt) being considered by MCDC.

### SPECIES CODES

BNT brown trout  
EEL eel  
LMB largemouth bass  
RBT rainbow trout  
SMB smallmouth bass  
WHP white perch  
WHS white sucker

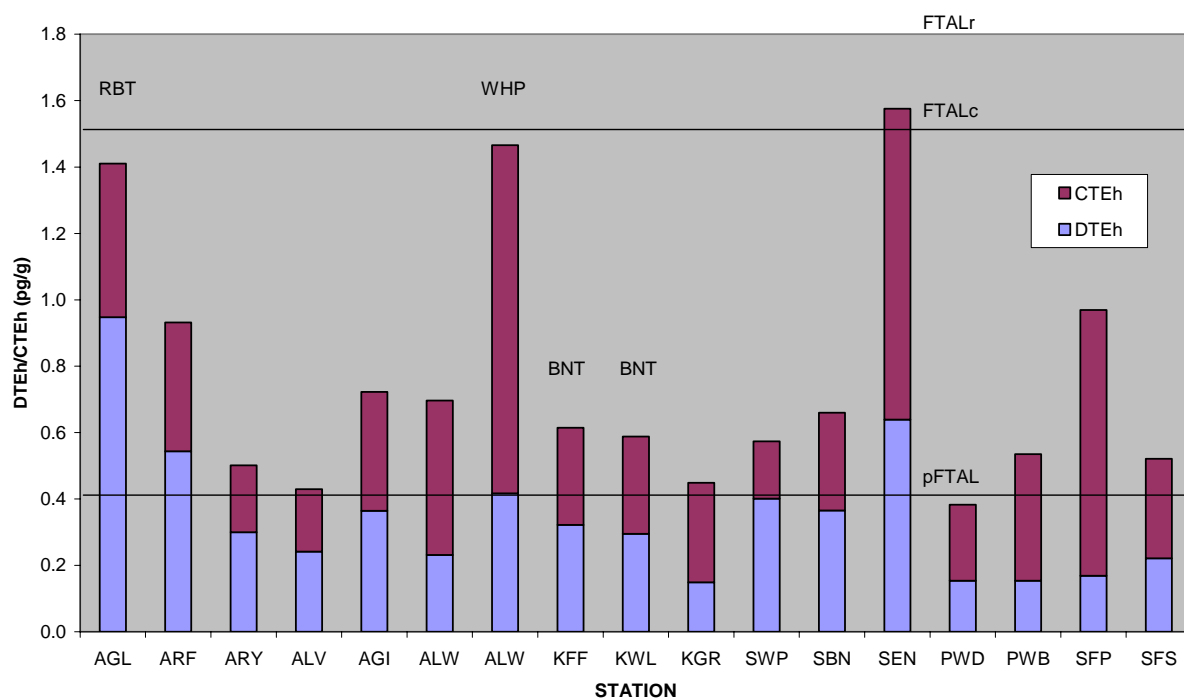
### STATION CODES

AGL Androscoggin R at Gilead  
ARF Androscoggin R at Rumford  
ARY Androscoggin R at Riley  
AGI Androscoggin R at GIP, Auburn  
ALV Androscoggin R at Livermore Falls  
ALW Androscoggin Lake at Wayne  
KFF Kennebec R at Shawmut, Fairfield  
PBW Penobscot R at Woodville  
PBL Penobscot R at S Lincoln  
PBV Penobscot R at Veazie  
SWP W Br Sebasticook R at Palmyra  
SBN Sebasticook R at Burnham

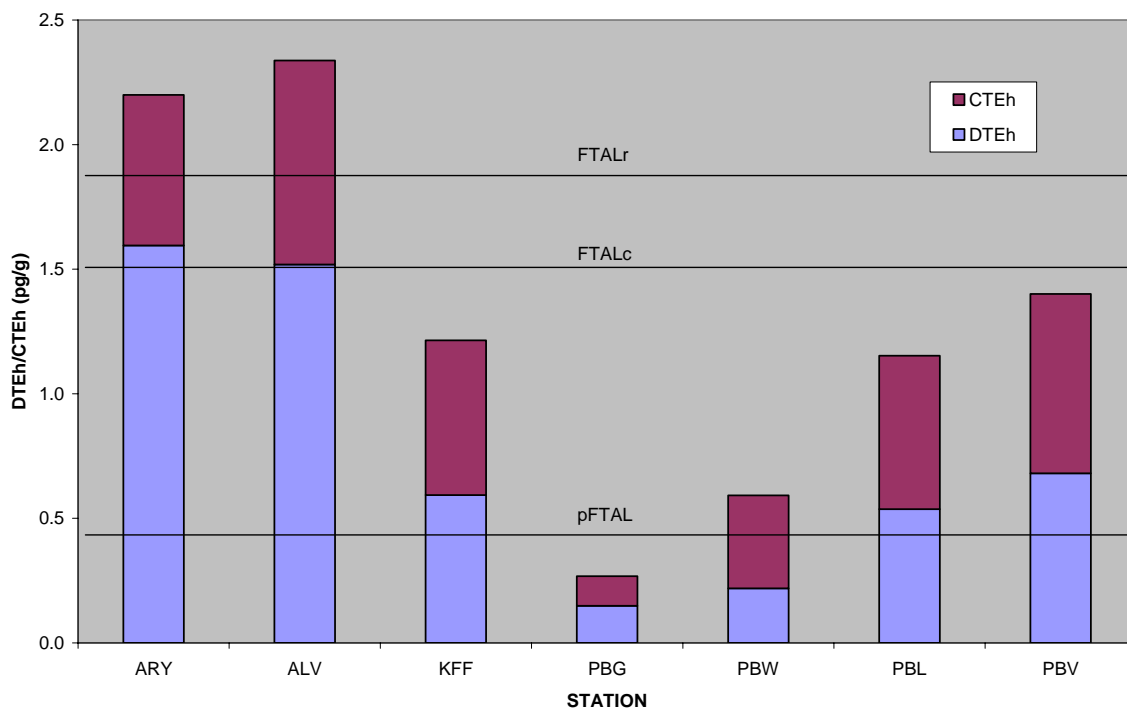
The results show that dioxin (toxic equivalents, upper 95% confidence limit with non-detects at ½ the detection level) and coplanar PCB (toxic equivalents, upper 95% confidence limit with non-detects at ½ the detection level) both separately and combined cause many samples to exceed the pFTALs and some to exceed the FTALc and FTALr (Figures 3.2.1 and 3.2.2, Appendix 3.2.1). The contribution of each varies with station, species and (not shown) year. Concentrations of coplanar PCB are within the wide range of those seen in previous years.

As in 2004 and 2005, coplanar PCB concentrations at SEN and ALW respectively were high. But as also in 2005, coplanar PCB at SWP were much lower than those in 2004, which were unusually high. Sources of PCBs are unknown but likely include atmospheric deposition.

**Figure 3.2.1. Dioxin (DTEh) and coplanar PCB (CTEh) toxic equivalents (95th UCL) in bass (and brown trout BNT, rainbow trout RBT, and white perch WHP) in the Androscoggin, Kennebec, Sebasticook, Presumpscot, and Salmon Falls rivers, 2006**



**Figure 3.2.2. Dioxin (DTEh) and coplanar PCB (CTEh) toxic equivalents (95th UCL) in white suckers from the Androscoggin, Kennebec, and Penobscot rivers, 2006**



## DIOXIN

Dioxin concentrations in rainbow trout at Gilead, in bass from two locations on the Penobscot River, and in sludge from the Somersworth New Hampshire municipal treatment plant were measured as part of the SWAT program but are discussed in detail in the 2006 final report of Maine's Dioxin Monitoring Program (DMP) available at <http://www.maine.gov/dep/blwq/docmonitoring/dioxin/index.htm> . Fish from selected stations from the Androscoggin River, Kennebec River, Presumpscot River, and Salmon Falls River were funded by other sources, and are also reported in the 2006 Dioxin Monitoring Program report.

## STRIPED BASS AND BLUEFISH

The current fish consumption advisory issued by the Bureau of Health for striped bass and bluefish recommends consumption of no more than 2 meals per month driven by total PCB concentrations. DEP had total PCB data from several years analyzed by various labs using some different methods. Beginning in 2003, samples have been analyzed for all 209 congeners. Data usually represent a mean of 5 individual fish.

There has been an increase in the concentration of total PCBs in striped bass and bluefish over the last three years. A significant effort is underway to revise these advisories and determine the feasibility of consistent advice from state to state along the east coast. One finding from that effort has been the need to sample striped bass from varying times to ensure different migratory stocks are being sampled. Up through 2005, striped bass were sampled during the spring fishery, but in 2006 samples were collected during the spring and fall for the Androscoggin and Kennebec rivers. We collected striped bass from the Saco River in the spring but were unable to collect any during the fall.

The upper 95<sup>th</sup> confidence level (95 UCL) mercury concentrations in striped bass in 2006 were higher than those from the other rivers and from previous years for the Androscoggin for both seasons (Table 3.2.1, Appendix 3.2.2). Concentrations for striped bass from the Kennebec River were similar to those of previous years for both seasons. Concentrations in the Kennebec striped bass were lower than found in freshwater fish from the same reach (not shown) and still exceeded the MDCDC's FTAL (0.2 ppm) for most samples. There were opposite trends between spring and fall samples for the two rivers, which is interesting since the Androscoggin is a tributary to the Kennebec. Also the Androscoggin striped bass were captured not far from the confluence and freshwater fish are more contaminated in the Kennebec River. The small sample size (n=3) makes any comparisons between seasons and rivers tenuous. Spring samples from the Saco River, were similar or slightly higher than the one previous sampling, but similar to those of the adjacent Scarborough River.

Table 3.2.1 Mercury in marine fish from Maine estuaries, mg/kg 95th UCL on the mean

striped bass	season	Androscoggin	Kennebec	Penobscot	Royal	Sheepscot	Saco	Scarboro	York
Year									
1995	spring		0.35						
1997	spring		0.33						
1998	spring	0.38	0.40					0.37	
1999	spring		0.32						
2000	spring	0.22				0.22	0.18		
2001	spring			0.15					0.12
2002	spring								
2003	spring								
2004	spring	0.24	0.23	0.32	0.17				0.21
2005	spring		0.28	0.44				0.28	0.15
2006	spring	0.56	0.35				0.30		
	fall	0.91	0.34						

The upper 95<sup>th</sup> confidence level (95 UCL) PCB concentrations in striped bass in 2006 were higher than those from the other rivers (Table 3.2.2, Appendix 3.2.2). Concentrations were similar to those from recent years for the Androscoggin for the spring season but lower than in recent years in the fall. Concentrations for striped bass from the Kennebec River were similar to those of previous years in the fall but lower in the fall. Concentrations in the Kennebec striped bass were lower than in recent years but similar to those found in freshwater fish from the same reach (Table 3.2.3). Concentrations still exceeded the MCDC's FTAL (11 ppb) for all samples. There were opposite trends between spring and fall samples for the two rivers, similar to the mercury data. The small sample size (n=3) makes any comparisons between seasons and rivers tenuous. Spring samples from the Saco River, were higher than previous data but similar to the most recent sampling of the adjacent Scarboro River.

Table 3.2.2 PCBs in marine fish from Maine estuaries, ppb mean (95 ucl on the mean)

striped bass	season	Androscoggin	Kennebec	Penobscot	Royal	Sheepscot	Saco	Scarboro	York
Year									
1995	spring		23 (30)						
1997	spring		11 (14)						
1998	spring	41 (43)	16 (17)				12.2	30.3	
1999	spring		11 (12)						
2000	spring	60 (72)				24 (28)	25 (32)		
2001	spring			84					64
2002	spring	288	93.2	279		149	135		103
2003	spring								
2004	spring	201	170	211	152				147
2005	spring		193 (269)	81 (110)				82 (262)	101 (108)
2006	spring	214 (429)	85 (98)				166 (228)		
	fall	88 (126)	114 (170)						

## KENNEBEC RIVER PCBs

The current advisory on the Kennebec River is no consumption of any fish from Augusta to the Chops. Limited consumption trout (5-6 meals per year) and limited consumption of bass (1-2 meals a month) from Shawmut Dam in Fairfield to Augusta. This advisory is based on a mix of contaminants. MCDC requested any fish from the river that were analyzed for dioxins and furans also be analyzed for coplanar PCBs, which was done as shown in figures 3.2.1 and 3.2.2. In addition, MCDC requested brown trout from Augusta to be analyzed for total PCBs, but none were captured. At the request of MCDC, smallmouth bass from Sidney, Augusta, and Gardiner were collected and analyzed for total PCBs to see if the trend continues downward from historically high levels responsible for the no consumption fish consumption advisory for the river below Augusta.

The results show that concentrations in smallmouth bass were perhaps slightly lower at Sidney and Augusta and similar at Gardiner to those of 2002, the most recent year sampled (Table 3.2.3). This may reflect washout of contaminated sediments since the Edwards dam was removed in 1999. Fish at Augusta have always been the most contaminated and are now showing the biggest change from previous data. Concentrations below Augusta remain elevated above the MCDC's Fish Tissue Action Level of 11 ppb.

Table 3.1.3 PCBs in smallmouth bass and brown trout from the Kennebec River  
ppb mean (95 ucl on the mean or max if n=2)

### Smallmouth Bass

Year	Norridgewock	Skowhegan	Fairfield	Sidney	Augusta	Gardiner
1994			4.5	8.6	604	
1997		3.7 (4.5)	4.0 (4.9)	6.1 (7.2)	342 (357)	
1999					263 (323)	179 (227)
2000				32 (42)		
2002	1.6		1.7	19.5	111	47.5
2006				7.5 (10)	83 (142)	51 (75)

## SALMON FALLS RIVER PCBS

The advisory on the Salmon Falls (based on data from S. Berwick) has been based on a few data (PCBs and dioxins). In 2006 largemouth bass were collected from the river in Spaulding Pond and the Somersworth impoundment in South Berwick and analyzed for total PCBs. Although historically there were discharges above and into Spaulding Pond, there are none known currently. The Berwick Sewer District discharge hosts the effluent from Prime Tanning Company and discharges above the South Berwick station. Results show that concentrations in Spaulding Pond are similar to that found upstream in Northeast Pond in 2002 (Table 3.2.4). Concentrations at South Berwick are slightly higher than the upstream stations but lower than in previous years at South Berwick, although the species sampled among the years are different. Concentrations in all samples exceed MCDC's FTAL (11ppb).

Table 3.2.4. PCBs in largemouth bass (LMB), smallmouth Bass (SMB), chain pickerel (CHP), and white perch from the Salmon Falls River, ppb mean (95 ucl on the mean)

Year	Acton	Northeast P	Spaulding P	Berwick	S. Berwick	
1997	5 (6)				75	SMB
					47 (53)	CHP
2000					83 (100)	SMB
2002		23.4	WHP	110		SMB
2006			25.5 (49)		33.2 (44)	LMB



### **3.3**

## **CUMMULATIVE EFFECTS DRIVEN ASSESSMENT OF FISH POPULATIONS**

## CUMMULATIVE EFFECTS ASSESSMENT OF FISH POPULATIONS

### Introduction

The US Clean Water Act (CWA) and Maine statutes set an ultimate goal that point source discharges be eliminated where appropriate and an interim goal that all waters be 'fishable/swimmable'. Maine Water Quality Standards further require that all freshwaters be 'suitable for the designated uses of ...fishing and ....as habitat for fish and other aquatic life' and be 'of sufficient quality to support ...indigenous species of fish'. EPA and DEP interpret 'fishing' to mean that not only do fish have to be present, but also healthy and safe to eat in unlimited quantities. And in order to provide 'habitat... to support a species', water quality must ensure that the population is sustainable, by allowing adequate survival, growth, and reproduction.

In the past, most SWAT studies of fish have focused on measuring the effects of persistent, toxic, and bioaccumulative (PBT) contaminants on human consumers, i.e. assessment of attainment of the designated use 'fishing', with some consideration of impacts to wildlife consumers as well. Direct effects on fish populations have been measured or estimated by other DEP programs able to detect only relatively severe impacts on survival, growth, and reproduction. Several studies (Adams et al, 1992; Kavlock et al, 1996; Munkittrick et al, 1998; Rolland et al, 1997) have measured other more subtle effects on development, immune system function, and reproduction not normally seen in more typical stressor-based testing regimes historically used by DEP. These more subtle effects may be a result of long term or cumulative exposure to relatively low levels of contaminants. These responses to pollutant challenge are often within the same magnitude as natural variation and therefore difficult to measure with the methods that are currently used. Many new techniques, such as a cumulative effects-driven assessment (CEA) of fish populations have been developed to measure some of these effects.

A CEA measures indicators of survival, growth, and reproduction. Age structure and mean age are measured as indicators of survival. Measures of energy expenditure and storage are used as indicators of growth and reproduction. Energy expenditure measures include size and size at age as indicators of growth while gonadosomatic index (GSI), fecundity, and egg size as indicators of reproductive potential. Energy storage measures include condition factor (K) as an indicator of growth and liversomatic index (LSI) and lipid storage as indicators of both growth and reproductive potential (Munkittrick et al, 2000). Response patterns of all indicators provide an integrative assessment of overall performance that may reflect different types of stresses, such as exploitation, food limitation, recruitment failure, niche shift, metabolic disruption (Munkittrick et al, 2000). Levels of circulating sex steroids are also often used as biomarkers of reproductive potential, which, along with survival, is considered an index of potential population trends.

With the assistance of Environment Canada (EC), DEP has conducted CEAs of fish populations on the St John River in 1999-2001 that have indicated probable impacts to fish populations and identified a previously unknown source. In 2000 similar studies of the North Branch of Presque Isle Stream and Prestile Stream, where high concentrations of DDT, a known endocrine disruptor, have been previously found, indicated a potential population level effects as indicated by a significant reduction in gonad size in both streams compared to two reference streams with much lower DDT levels in fish.

For Maine's major industrial rivers, the initial plan was to study what was considered the worst case first, and if no negative impacts were measured not to study the other rivers. The Androscoggin River was chosen to study first among the large industrial rivers because it had more (3) large pulp and paper mills for its size than the other major rivers and has historically had the poorest water quality. CEAs of white sucker populations in the Gulf Island Pond on the Androscoggin River from 2001-2003 did not show the evidence of endocrine disruption and metabolic redistribution found in a preliminary study in 1994. This result is possibly due to the change in bleaching technology from free chlorine to chlorine dioxide and improved waste treatment in the 3 upstream bleached kraft pulp and paper mills in the intervening years. Nor was there any evidence of endocrine disruption at any location below any of the mills in the rest of the river. There was evidence of increased eutrophication that correlated with increased nutrient levels downstream of the mills and host municipalities (DEP, 2004).

Studies of caged mussels in 2003 on the Androscoggin River showed no negative impacts on growth rate or induction of vitellin, a reproductive protein biomarker of endocrine disruption. This result is consistent with the CEA of fish populations in the river from 2001-2003. Studies of caged mussels in 2003 on the Kennebec River, however, did show induction of vitellin below a bleached kraft pulp and paper mill, evidence of endocrine disruption. Therefore, in 2004, a CEA was conducted on white suckers above and below the SAPPI Somerset bleached kraft pulp and paper mill on the Kennebec River. The results indicated possible endocrine disruption of survival, growth, and reproduction, as mean age, length in males, and levels of circulating sex steroids were reduced below the mill. Yet the results were not conclusive since MFOs, an indicator of exposure to bleached kraft mill effluent and other xenobiotics, and LSI in females were reduced below the mill while GSI and K actually increased, indicating a shift in energy storage and utilization and/or nutrient enrichment. This study was repeated in 2006.

A caged mussel study in 2004 did not show induction of vitellin seen in 2003, but the stations were different between the two years due to other priorities. The study was repeated in 2005 and showed induction of vitellin, below the mill as in 2003, although at station 5 rather than stations 3 and 4, for both males and females. In addition, lipid peroxidation, an indicator of toxicity, was elevated at all three stations below the mill. Growth in length and/or weight was increased at all stations below the mill. This study was repeated in 2006 but the data are not yet available from the lab.

Since there is some evidence of endocrine disruption below the bleached kraft mill on the Kennebec River, in 2005 a CEA was conducted above and below the Lincoln Paper and Tissue bleached kraft mill on the Penobscot River. Fish samples were collected in conjunction with the dioxin above/below (A/B) test, which allowed sampling effort and use of fish for both studies. The Environmental Effects Monitoring (EEM) program in Canada require all pulp and paper mills to conduct CEA of two species for each mill. Our 2005 Penobscot River CEA included two species as well, smallmouth bass and white sucker. Also a caged mussel study was initiated for the Penobscot for the A/B dioxin test and measurement of vitellin, but heavy fall rains and subsequent flooding prevented retrieval of the mussels.

Many studies have also documented effects of heavy metals, PAHs, sewage, and pulp and paper mill waste on fish immune systems (Voccia et al, 1994; Holliday et al, 1998; Secombes et al, 1992; Ahmad et al, 1998). We have measured the spleen somatic index (SSI) and kidney somatic index (KSI) from white suckers from the Androscoggin River from 2002-2003, the Kennebec River in 2004, and Penobscot River in 2005 as rough indicators of immune system effects. There were significant decreases in SSI below the 2 most upstream mills on the Androscoggin for one or both sexes in 2002

and 2003, indicating potential immune system stress. Similarly, SSI was decreased below the SAPPI Somerset bleached kraft mill on the Kennebec River in 2004 not inconsistent with the possible decreased immune system capacity found by Hannum in head kidneys (SWAT, 2004), although the mechanism is unclear since head kidney size (KSI) in our study was no different between sites above and below the mills for either sex on either river. Both SSI and KSI were measured on both species from the Penobscot River in 2005.

## Methods

In September 2006, white suckers were collected from the Kennebec River at Norridgewock (KNW) and Fairfield (KFF) above and below the discharges from the City of Skowhegan and the SAPPI bleached kraft pulp and paper mill. Similar sampling was conducted on the Penobscot River at 4 stations. The stations were 1) above Millinockett on the West Branch of the Penobscot River in the Nesowadnehunk deadwater (PBN), 2) above East Millinockett on the East Branch of the Penobscot River above Grindstone (PBG) 3) below Millinockett and East Millinockett (with 2 municipal and 2 pulp and paper mill discharges) and the confluence with the East and West branches of the river mill (PBW) at Woodland, above the Mattaceunk dam and above the Lincoln Paper and Tissue mill and 4) downstream of the Lincoln mill at South Lincoln (PBL), approximately 3 miles below the mill at the historic sampling site for the Dioxin Monitoring Program. For each of the stations, 20 males (except PBG where no males were collected) and 20 females of each species were collected during fall recrudescence. Previous studies have determined that a sample size of 20 is sufficient to reduce the variance enough to detect a difference of 20-30% in the variables measured between stations.

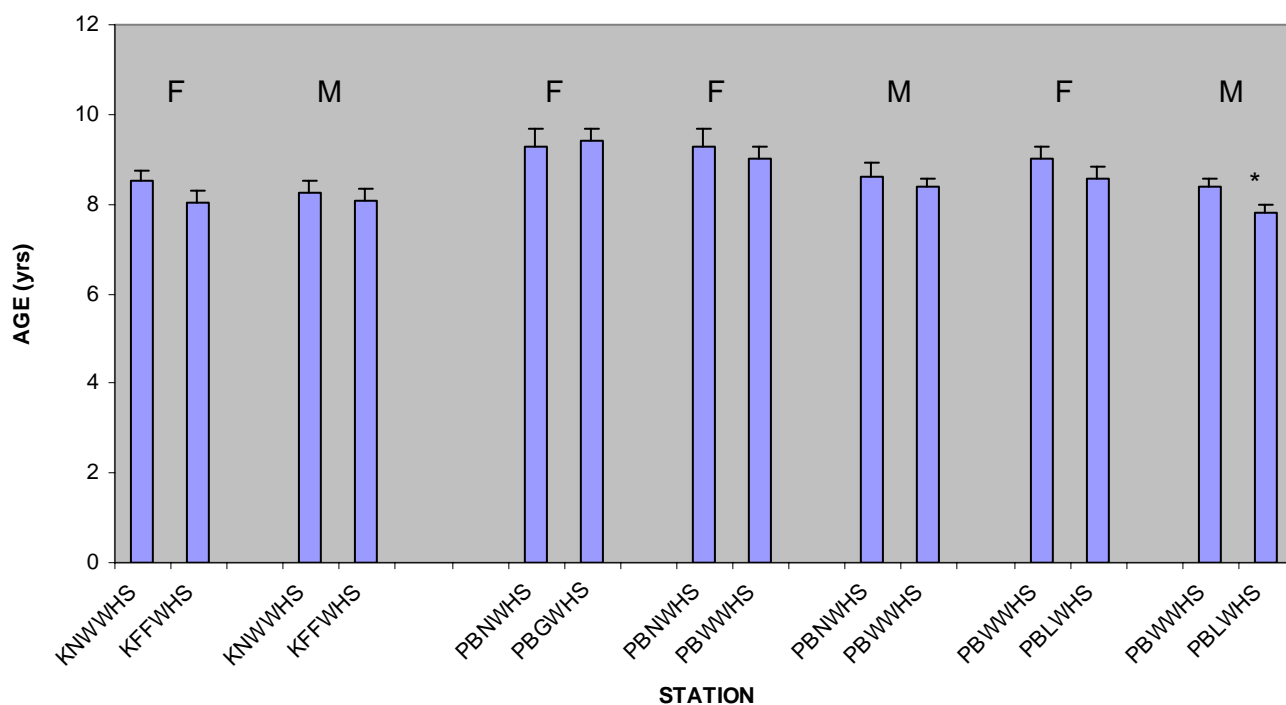
Fish were collected by gill net. Blood samples were collected from live fish immobilized in a foam cradle, into heparinized Vacutainers and placed on ice for transport to the lab the same day. The fish were then killed with a blow to the head. The operculum was collected for aging. Livers were dissected out and weighed, for calculation of LSI, and then frozen in liquid nitrogen. Gonads were dissected out and weighed for calculation of GSI and a small sample ~1 cm square was taken and placed in 10% buffered formalin for storage. Head kidney in suckers and spleen in both species were dissected out and weighed for calculation of KSI and SSI respectively.

Later the same day in the lab, the samples were placed in proper storage to await analyses. Plasma was collected from the blood samples after centrifugation in the lab and then frozen at -20C for radioimmunoassay (RIA) analysis for circulating sex steroids (testosterone T, 11 ketotestosterone 11-KT, and estradiol E2) following the method of McMaster et al (1992) and F following the method of Jardine (1996). Liver samples were stored at -80 C for MFO (CYP1A) analysis as outlined by Munkittrick et al (1992). Gonad samples remained in formalin for further analyses. Histological samples of gonads were prepared and examined for the presence of testis-ova as outlined in Gray and Metcalf (1997) or analysis of gonadal staging (McMaster, 2001). All laboratory analyses were performed by at Environment Canada's National Water Research Institute in Burlington, Ontario, Canada. Samples for aging were stored at -20C until prepared and read in the DEP lab in Augusta, Maine.

## Results

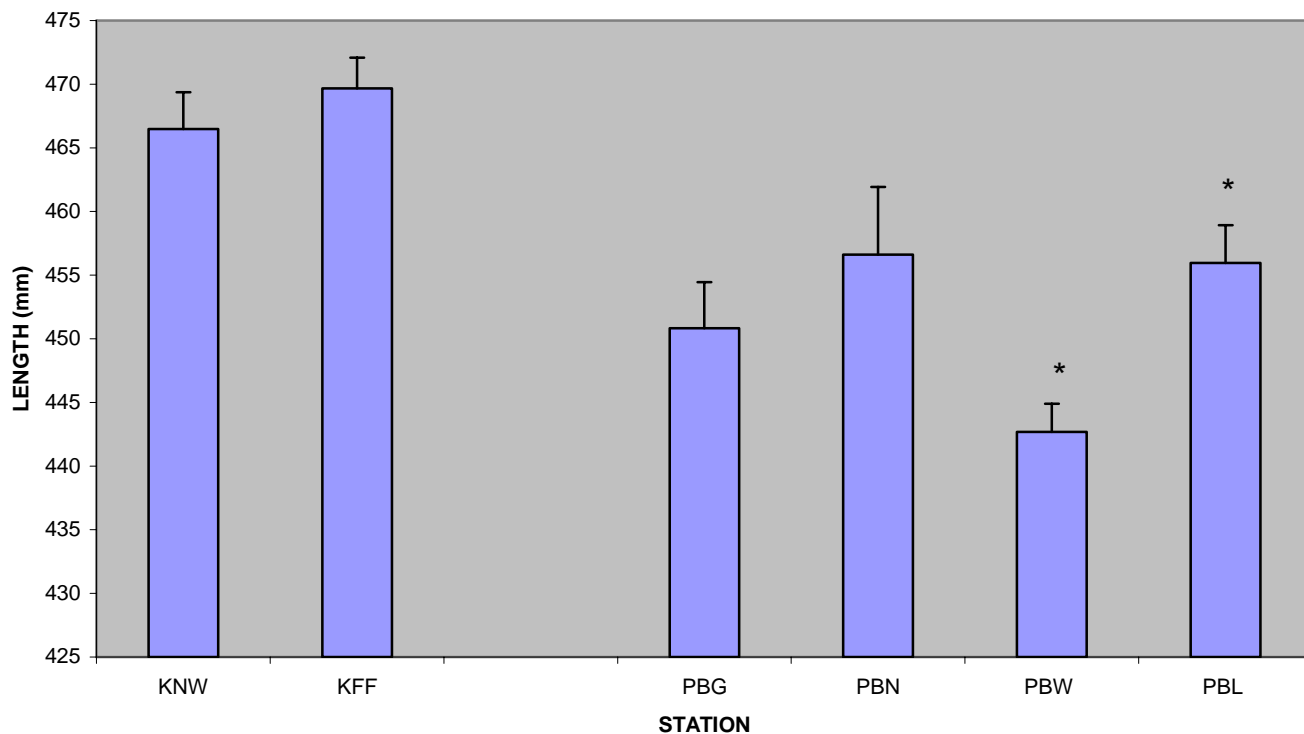
There was no difference in mean age, an indicator of survival, above and below the mill on the Kennebec River, unlike in 2004 when mean age was lower below the mill for both sexes (Figure 3.3.1). Nor was there any difference in mean age among any of the 3 stations above Lincoln on the Penobscot River. Mean age was significantly reduced in male white suckers from the Penobscot River below the mill at PBL compared to above the mill at PBW, unlike in 2005. Munkittrick (2000) gives as two possible reasons for reduced survival, 1) exploitation and 2) metabolic redistribution. Few people angle recreationally for white suckers and there is no one known to be netting white suckers for lobster bait in this reach, so exploitation is considered to be nil.

**Figure 3.3.1. MEAN AGE OF MALE (M) AND FEMALE (F) WHITE SUCKERS SAMPLED FROM THE KENNEBEC AND PENOBSCOT RIVERS, 2006**



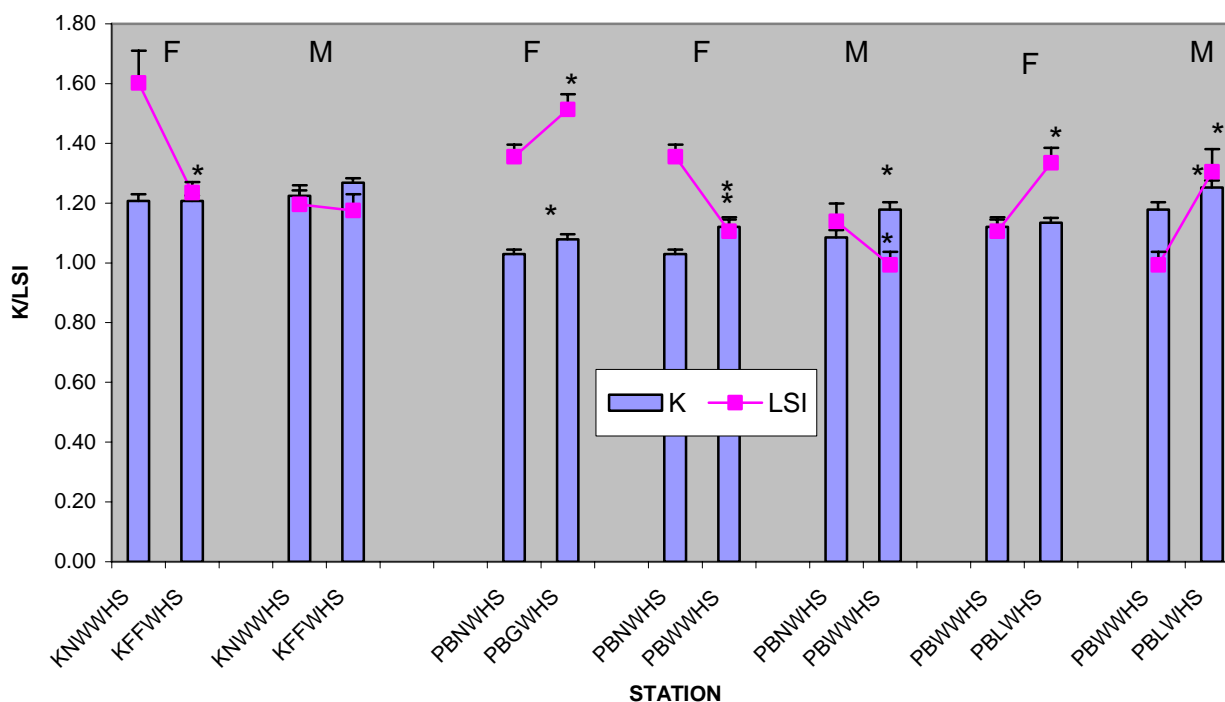
Indicators of growth include measures of energy expenditure (size and size at age) and measures of energy storage (condition factor K and liversomatic index LSI). Mean size as measured by length was biased in the 2005 study since the white suckers were also used for dioxin analysis which required that the fish be selected for a relatively uniform length. Therefore, in 2006 all fish captured were measured for length. Sample sizes were variable (PBN n=38, PBG n=28, PBW n=137, PBL n=100). Mean length was lower at PBW than any of the other stations (Figure 3.3.2). PBW is downstream of Millinockett where there are 2 municipal treatment plants and 2 pulp and paper mills. Increased length combined with decreased age below Lincoln implies increased growth, probably due to nutrient enrichment as was the case in 2005 for bass although not for suckers. There was no difference in length for white suckers on the Kennebec above and below the mill, unlike in 2004 when males were smaller below the mill.

**Figure 3.3.2. MEAN LENGTHS OF WHITE SUCKERS FROM THE KENNEBEC AND PENOBSCOT RIVERS, 2006**



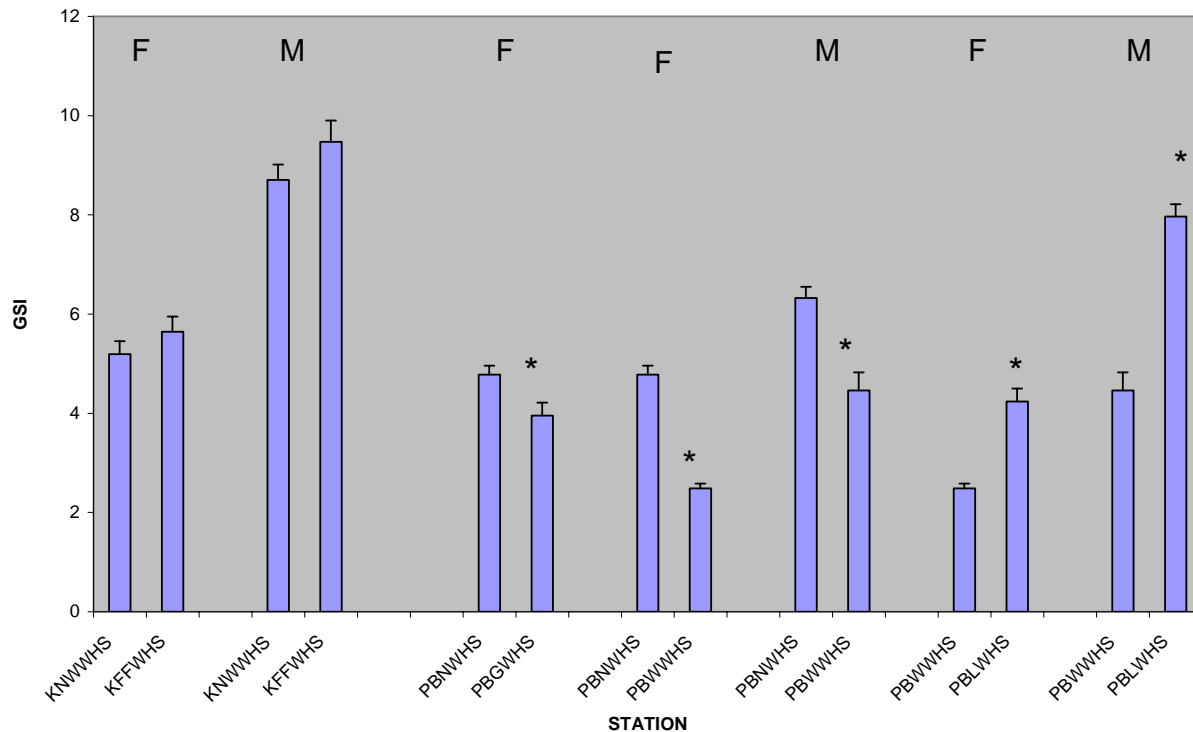
Unlike in 2004, there was no increase in K for either sex below the SAPPI mill at KFF in 2006 (Figure 3.3.3). LSI was similar to that of 2005 with a decrease in females at KFF and no change in males. Both metrics, then, indicate a loss or no increase in energy storage, unlike 2004. In the Penobscot both K and LSI increased below the Lincoln mill at PBL in both sexes, except for K in females, as was the case in 2005, indicating continued energy storage.

Figure 3.3.3. MEAN K AND LSI OF MALE (M) AND FEMALE (F) WHITE SUCKERS SAMPLED FROM THE KENNEBEC AND PENOBSCOT RIVERS, 2006



Indicators of reproduction also include measures of energy expenditure (gonadosomatic index (GSI), fecundity, and egg size) and measures of energy storage (LSI and lipid storage). Unlike in 2004, there was no increase in GSI at KFF below the SAPPI mill on the Kennebec River (Figure 3.3.4). For the Penobscot River, GSI increased at PBL below the Lincoln mill in both sexes as in 2005. Interestingly, suckers at PBW had lower GSI than the two stations upstream of Millinocket, PBN and PBG.

**Figure 3.3.4. MEAN GSI OF MALE (M) AND FEMALE (F) WHITE SUCKERS FROM THE KENNEBEC AND PENOBSCOT RIVERS, 2006**

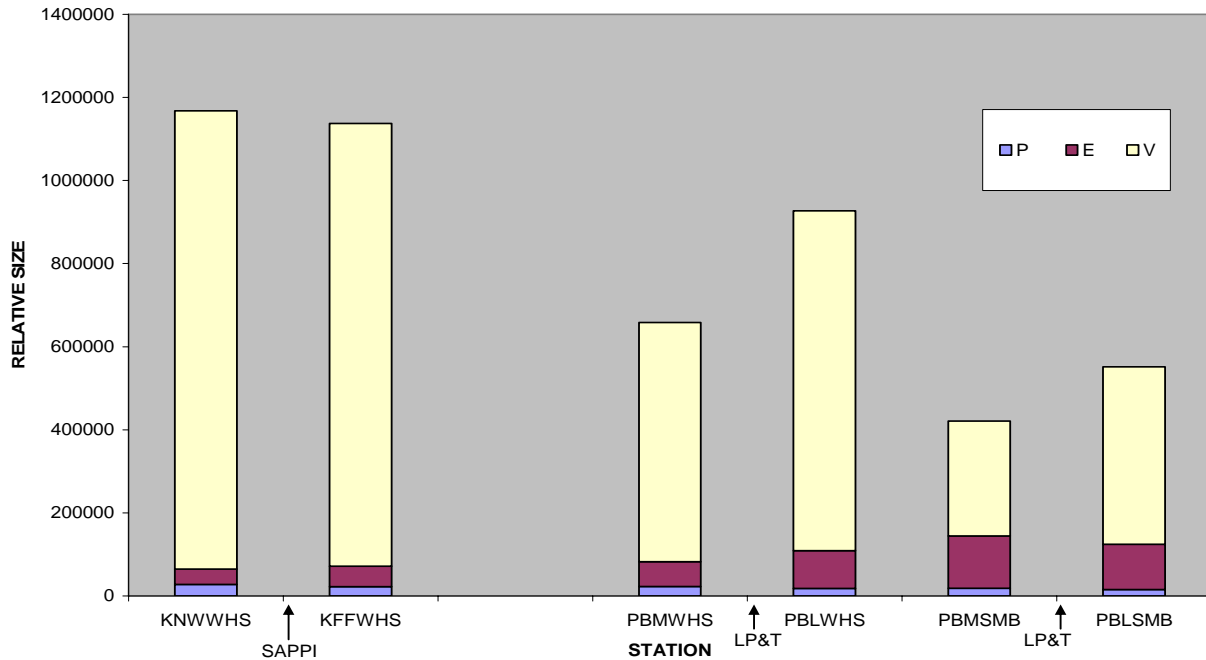


Data for gonad samples of white suckers from the Kennebec River in 2004 and the Penobscot River in 2005 have been recently received and are first reported here. Testes were examined for intersex, i.e. the inclusion of ova in the testes (ovo-testis). Ovaries were examined for gonadal staging, i.e. developmental stage of oocyte, and were classified as primary (pre-vitellogenic), endovitellogenic (endogenous vitellogenic), or vitellogenic.

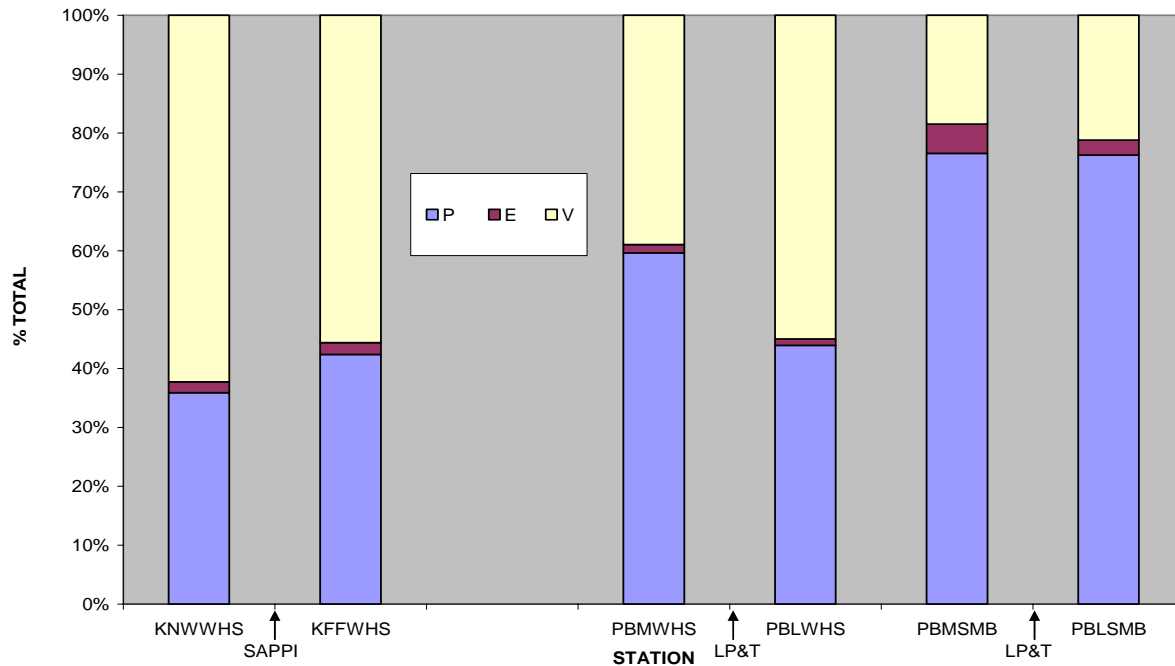
There were no males reported to have intersex for the Kennebec River fish. Results of the gonadal staging show no difference in mean size or percent of primary, endovitellogenic, or vitellogenic oocytes (Figures 3.3.5 and 3.3.6). Results show that for the Penobscot River there were 2 intersex males (out of 20) below Millinockett at PBW and 2 intersex males (out of 20) below Lincoln at PBL. This 10% incidence is not unusually high compared to reports from the literature, but the significance is not certain at this time. Gonadal staging did show some differences above and below the mill. In white suckers, vitellogenic oocytes were significantly larger below the mill (Figure 3.3.5) and there was advanced development as evidenced by a shift from percent primary to vitellogenic oocytes there as well (Figure 3.3.6). For smallmouth bass vitellogenic oocytes were also larger below the mill but there was no advanced development.



**Figure 3.3.5. Mean size of primary (P), endovitellogenic (E) and vitellogenic (V) oocytes in female white suckers from the Kennebec River and the Penobscot River, 2006**



**Figure 3.3.6. Mean percentage of total as primary (P), endovitellogenic (E), and vitellogenic (V) oocytes in female white suckers from the Kennebec and Penobscot rivers, 2006**



## Conclusions

For the Kennebec River, field data responses seen in 2004 were generally not repeated in 2006. The only response that was consistent between both years was an increase in energy storage in the liver, i.e. increased LSI, of white suckers below the SAPPI mill. The increased energy utilization, i.e. increased growth in females and increased GSI in both sexes, measured in 2004 was not observed in 2006. However, the MFO, steroid, vitellogenin, and gonad data have not been reported from the lab yet, so final conclusions cannot be made at this time.

For the Penobscot River, responses were generally similar to those of 2005 for energy utilization as indicated by increased GSI and for energy storage as indicated by increased K for males and LSI for both sexes below the mill at PBL. Responses were also similar to those of 2005 with no change in growth and no increase in K in females. There were some differences from 2005, namely direction of energy to growth (size at age) in males. The MFO, steroid, vitellogenin and gonad data have not been reported from the lab yet, so final conclusions cannot be made at this time.

The 2005 gonad data from white suckers from the Penobscot River below Millinockett and Lincoln showed intersex in a small (10%) incidence, that is reported to be not uncommon in the literature. Gonadal staging showed some advanced development of oocytes below Lincoln as well. These data need to be compared to the 2006 data, when available, before the significance is known.

These responses for male white suckers in the Penobscot River fit a pattern of exploitation for 2006, caused by mortality or eutrophication. Since there is no directed fishery for white suckers on the Penobscot, these responses are more likely caused by eutrophication. Increased productivity of the system at PBL is likely due to nutrient discharges from the mill and treated municipal wastewater from the Town of Lincoln.

The responses also fit a pattern of metabolic disruption, but the steroid data are needed for a final determination. For suckers the pattern of responses most closely resembles one of metabolic disruption with energy directed mostly toward reproduction and little toward growth in females and both in males.

There was no difference in head kidney somatic index (KSI) or spleen somatic index (SSI) between PBM and PBL for males, but SSI increased in females below the mill at PBL in 2006 (Figure 3.3.7), unlike 2005 when there was no difference above and below the mill for either sex. This finding is unlike that from the Kennebec and Androscoggin rivers in previous years where SSI was significantly lower below the mills and host municipalities, both of which were larger than Lincoln on the Penobscot River.

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## 3.4

### Caged Mussel Vitellin Study

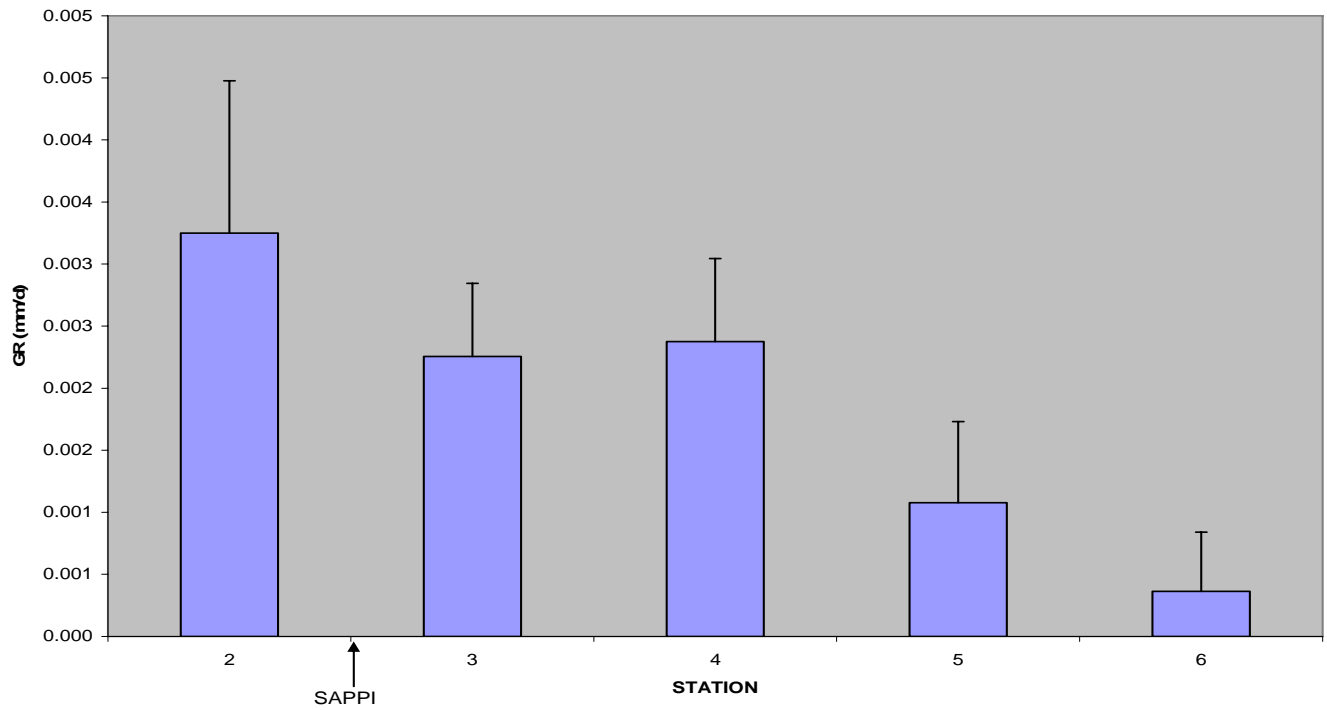
## CAGED MUSSEL VITELLIN STUDY -DEP

In 2003 a study with caged mussels detected a significant induction of vitellin, a vitellogenin-like reproductive protein normally found in females, in a subsample of both males and females at stations 3 and 4, ~ 0.08 miles and 2.5 miles below the SAPPI bleached kraft pulp and paper mill on the Kennebec River respectively compared to stations KR1 and KR2, ~ 13 and 5 miles above the mill respectively. Growth of whole animal length and weight, shell weight, and wet tissue weight were elevated at station KR5, ~ 5 miles below the mill. A repeat study in 2004 found no such induction at station KR6, ~ 11 miles below the mill, compared to KR2 and there was no difference in condition factor or relative gonad size (GSI) between the stations. A repeat study in 2005 found increased growth in shell length and whole animal wet weight at all stations sampled below the mill (KR3-5). ALP (vitellin) total mass was greater at KR5 than the upstream station KR2, but ALP normalized to protein was no different between the two. In 2005 there was a significant increase in lipid peroxidation at all stations below the mill. The study was repeated in 2006 including all stations 2-6. A similar study was attempted in the Penobscot River above (PBW) or below (PBL and PBC) the Lincoln Paper and Tissue bleached kraft pulp and paper mill at Lincoln in 2005, but samples were lost in a flood. The study was repeated in 2006.

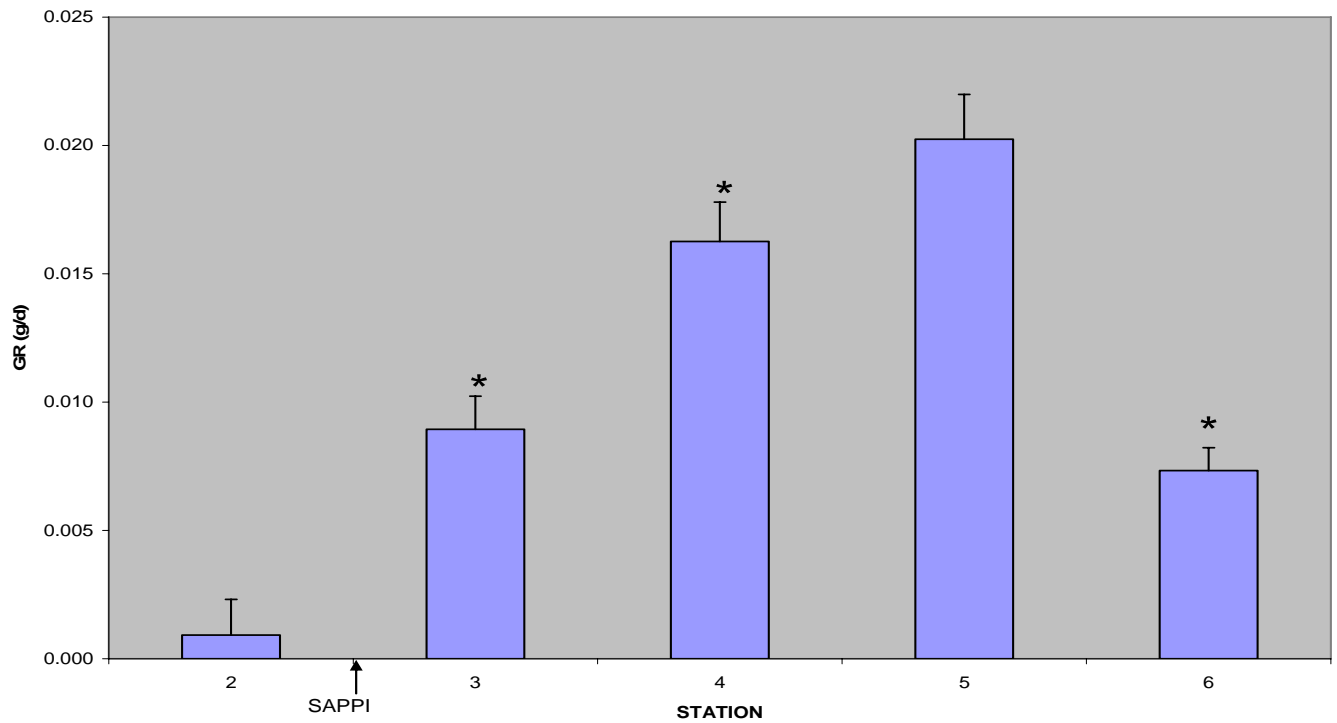
### Results

In the Kennebec River, unlike 2005, growth rates for length were no different among the stations (Figure 3.4.1). Growth rates for whole animal wet weight were significantly greater below the mill than above dropping off at KR6 (Figure 3.5.2) similar to that for KR5 in 2003 all stations below the mill in 2005. In the Penobscot River there was no difference in growth for either shell length or whole animal wet weight among the stations, due to high variability among the stations (Figures 3.4.3 and 3.4.4). The indication of enrichment in the Kennebec below the mill and not in the Penobscot below the mill was opposite that seen with the fish for both rivers. The vitellin and other lipid peroxidation data have not yet been received from the lab.

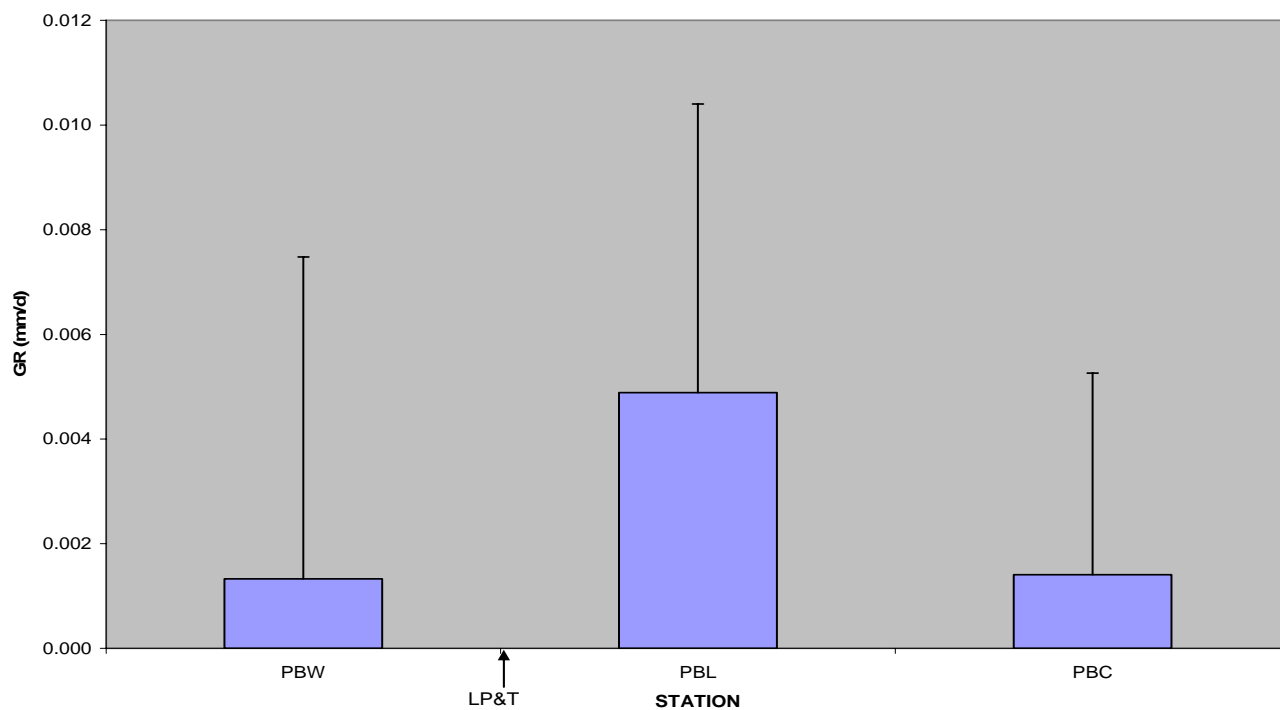
**Figure 3.4.1. Growth rates in length of caged mussels in the Kennebec River, 2006**



**Figure 3.4.2. Growth rate in weight of caged mussels in the Kennebec River, 2006**



**Figure 3.4.3. Growth rate in length of caged mussels in the Penobscot River, 2006 (mean+se)**



**Figure 3.4.4. Growth rate in weight of caged mussels in the Penobscot River, 2006 (mean+se)**

